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• *Original Contributions*

MALE REPRODUCTIVE EFFECTS OF SOLVENT AND FUEL EXPOSURE DURING AIRCRAFT MAINTENANCE

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Abstract — Few studies have addressed the effects of mixed, low-level exposures to complex mixtures on a man's reproductive potential. In this prospective study, each subject was evaluated before first exposure and at 15 and 30 weeks after exposures had begun. A total of 50 men working on aircraft maintenance at an Air Force installation were included in the study. In addition, eight unexposed men were concurrently sampled. Industrial hygiene (IH) sampling and expired breath samples were collected for jet fuel as measured by total napthas, benzene—a component of jet fuel, 1,1,1-trichloroethane, methyl ethyl ketone, xylenes, toluene, and methylene chloride. Sperm production, structure, and function (sperm concentration, sperm motion, viability, morphology, morphometrics, and stability of sperm chromatin) were evaluated. Exposures were low. All mean IH measures were below 6 ppm, which is less than 10% of the Occupational Safety and Health Administration standard for all chemicals except benzene. Sheet metal workers had the highest mean breath levels for both total solvents (24 ppb) and fuels (28.3 ppb). For most sperm measures, mean values remained in the normal range throughout the 30 weeks of exposure. When jobs were analyzed by exposure groups, some adverse changes were observed. The paint shop group had a significant decline in motility of 19.5% at 30 weeks. Internal dose measures, however, did not show a significant association with spermatogenic changes. © 1999 Elsevier Science Inc.

Key Words: mixtures; fuels; solvents; male reproduction; sperm; painters; aircraft maintenance; breath analysis.

INTRODUCTION

The past decade has seen growing concern that environmental exposures pose threats to male reproductive health, as about half of the drugs and chemicals evaluated in human semen analysis have been shown to reduce sperm quantity or quality (1). Furthermore, testicular cancer has increased and has been linked, in part, with exposure to fertilizers, phenols, and fumes, or smoke (2). There is evidence that the ratio of male to female births is declining in several industrialized countries (3). This decline has been linked to dioxins (4), anesthetic gases (5), pesticides (6–10), occupational exposures in the aluminum industry (11), and exposures to lead and solvents (12). Others, however, have found that the male to female sex ratio has declined only among Caucasians and not African-Americans, which raises doubts of an environmental explanation of effects (13). One controversy continues concerning report of a gradual decline in sperm count over the last 50 years that is suspected to have links with environmental exposures (14–18).

The potential reproductive effect of exposure to solvents and fuels is currently under scrutiny because of their widespread use. It has been estimated that more than 100 million people are at least briefly exposed to volatile organic compounds (VOC) on a regular basis (19). Common VOCs are solvents, found in a variety of degreasing agents, paints, coatings, pesticides, and strip-

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pers. Fuels are complex mixtures of hydrocarbons containing more than 1,000 substances and gasoline, diesel fuel, jet fuel, and the products of their complete and incomplete combustion are among the sources of fuel exposure encountered (20). It was recently shown that, even during a short period of pumping gasoline, blood VOCs for benzene, ethyl benzene, xylene, and toluene significantly increased (21). Glycol ethers are widely used VOCs, and studies of rats, mice, rabbits, and dogs demonstrate an association of 2-ethoxyethanol (2EE) with a variety of adverse outcomes, including testicular atrophy, degeneration of seminiferous tubules, severe oligospermia, abnormal sperm morphology, and reduced sperm motility (22–25). Two studies indicate a significant association of occupational exposure to 2EE and decreased sperm count at both a metal castings company (26) and in painters (27). Perchloroethylene is another VOC with particular use as a dry cleaning agent; a study comparing 34 dry cleaners with 48 laundry workers identified significant dose-related changes in sperm morphology and sperm movement (28). Other studies of mixed solvent exposures found a decrease in follicle-stimulating hormone (FSH) (29) and male-mediated effects such as an increase in spontaneous abortions and congenital malformations among the wives of men exposed to fuels, glycol ethers, toluene, and xylene (30–32). There was, however, no decrease in the birth rates among partners of men occupationally exposed to glycol ethers in the semiconductor industry (33).

The objective of this prospective epidemiologic study was to examine the association of work on aircraft maintenance operations with exposure to fuels and solvent mixtures, and the biologic effects on the male reproductive system, as well as cytogenetic effects on blood lymphocytes. The solvents studied included 1,1,1-trichloroethane (TCA), toluene, methyl ethyl ketone (MEK), xylene, and methylene chloride. This study categorized subjects performing aircraft maintenance into job groups using personal industrial hygiene (IH) monitoring to augment job descriptions. Internal dose levels of the toxicants were measured by monitoring expired breath samples and were then linked to early biologic effect markers measuring genotoxic and spermatotoxic effects. The results of the genotoxicity study have been reported previously (34). Briefly stated, a small but statistically significant increase was found in sister chromatid exchanges and micronuclei by 30 weeks of exposure for aircraft maintenance personnel performing sheet metal operations and painting activities. This report examines whether low-level exposure to solvents and fuels are associated with changes in semen parameters from baseline (pre-exposure) to 15 and 30 weeks after job initiation.

STUDY DESIGN AND SUBJECT SELECTION

Data were collected using a prospective, repeated measures design with each subject serving as his own control. In addition, eight unexposed subjects were recruited to examine the potential for secular or seasonal trends and assay variability in men without any occupational exposure. All study subjects were volunteer civilian or active-duty military personnel at one U.S. Air Force base who were 51 years of age or younger. The screening questionnaire was administered to all potential participants to determine eligibility by collecting detailed information about all previous jobs and hobbies. Anyone with recent (prior 12 months) exposure to chemicals known to be spermatotoxic or mutagenic was excluded.

Outcomes were assessed for three cycles: Cycle 1 was a baseline taken before entry into the exposed job; Cycles 2 and 3 were at 15 and 30 weeks during occupational exposure to solvents and/or jet fuels (primarily JP4). Though these intervals were chosen to allow for two complete spermatogenic cycles, it is recognized that in the distal regions of the human epididymis there is widely differing sperm age (35). During the baseline period (before entry into the work place) semen samples were collected but no exposure monitoring was performed. For Cycles 2 and 3, semen assays and exposure assessment (e.g., industrial hygiene monitoring and breath sampling) were collected for the exposed group and only semen assays were requested from the nonexposed office workers.

Questionnaire Construction and Administration

Three questionnaires were used in this study: 1) a screening questionnaire, 2) a background medical and occupational history, and 3) a cycle update questionnaire. Questionnaires were pretested during the pilot phase of the project (36) and administered face-to-face. The background medical and occupational history included life-style characteristics (e.g., exercise frequency, smoking, alcohol, and caffeine intake), background health history (chronic diseases, birth defects, urogenital disease, and medication use), and details of past and current work histories. The cycle questionnaire updated information at 15 and 30 weeks to assess recurrent fevers, illness, medical procedures, prescription and nonprescription drug use, alcohol, caffeine, and tobacco use, as well as changes in occupation, hobby, or home exposures.

Exposure Monitoring

Work place monitoring used standard personal IH sampling procedures in accordance with National Institute for Occupational Safety and Health (NIOSH) guidelines (37). During Cycles 2 and 3, subjects received three

full 8-h shifts of IH measurement on consecutive days, with breath sampling on the third day. At other times outside the scheduled periods for biologic sampling, unannounced IH samples were collected to ensure representativeness of exposures. Each sample was analyzed for the aforementioned targeted compounds. Values below the limit of detection (LOD) were replaced with the LOD divided by the square root of two according to Hornung et al. (38).

Internal dose, or body burden, was assessed using expired breath because: 1) in our pilot study, it was found to be the most sensitive dose measure compared with blood and urine (36), 2) solvents and fuels are primarily absorbed by percutaneous and inhalation routes and excreted through exhalation (39–44), and 3) this method is noninvasive. After a normal work shift, subjects waited for one hour prior to breath sample collection in a separate building having no chemical exposures (the clinic). This 1-h period allowed time for steady state lung ventilation to be achieved to more closely approximate blood levels rather than the most recent exposure that is captured immediately post exposure. The sampling apparatus was a modified version of the system developed by Conkle et al. (45) for the Air Force. Zero grade air (maximum 1 ppm of total hydrocarbons) was supplied to the subjects through low resistance, 1-inch-diameter stainless steel air ducts using a mouthpiece with two one-way valves. Samples were collected on Tenax tubes for 20 min at 1 L/min.

Reproductive Assays

The biologic effects of solvent exposure were measured by reproductive assays at each cycle. Subjects were briefed, given written instructions and a NIOSH video on sample collection procedures, and a 48-h abstinence period was requested before semen sample collection. In workplace studies, home collection of semen samples is considered more feasible compared with a clinical setting where on-site semen samples are often requested (46). Therefore, samples were collected by masturbation at home and transported within 45 min in a warmed thermos. Time of collection and arrival, liquefaction status, volume, and color were recorded, along with days of abstinence and amount of sample spilled, and pH and temperature were measured using an electronic pH/temperature meter.

Sperm concentration and count, and percent viable by both stain exclusion and hypoosmotic stress were performed on the fresh specimens. To determine sperm concentration, an aliquot of semen was thoroughly mixed with chloramine T solution to kill the sperm. The suspension was loaded into the two chambers of a 20-μ Microcell slide, and the number of sperm in the 5×5 ocular grid was counted on both sides of the Microcell. If the two sides differed by more than 20%, a second dilution was prepared and the final sperm concentration was computed from the median of the three counts. Videotaping of the semen was initiated at approximately 1-h post-collection. One side of a 20-μ Microcell was loaded with undiluted semen and the other with semen diluted in warmed (37°C) Dulbecco's phosphate-buffered saline. The amount of saline used for the dilution was based on the sperm concentration and was calculated to yield 40 to 80 million sperm per mL. Microcell chambers were maintained at 37°C during videotaping using a microscope stage warmer while sufficient fields were videotaped to evaluate 200 sperm cells. Percent motile, sperm velocities, and swimming pattern characteristics were analyzed using the CellTrak/S Research computer-assisted sperm analysis system (CASA) from Motion Analysis Corp. The stain exclusion test for viability used eosin Y stain, and stained and unstained cells were differentially counted for a total of 200 sperm. The hypoosmotic swelling test for viability was conducted by mixing a semen aliquot in equal parts 150 mOsm fructose and 150 mOsm sodium citrate. The mixture was incubated at least 30 min at room temperature.

Additional semen analyses were performed by investigators at other laboratories. Samples were coded to ensure investigators were unaware of the subject's exposure status. Samples were evaluated in batches across cycles. Four air-dried semen smears were made on microscope slides. Two were stained with Papanicolaou stain for objective morphometry. The other pair were stained with the Feulgen reaction for objective morphometry. Both traditional and strict criteria were used to determine percent normal morphology and morphometry. Semen specimens were prepared for sperm chromatin structure assay (SCSA) using standard methodology as described elsewhere (47,48). Green and red fluorescence values for each sample were used for calculations of the mean, standard deviation, and percent of cells demonstrating DNA denaturation. The SCSA has been associated with exposure to genotoxicants and male infertility (49,50).

Creatine phosphokinase (CK) activity is a key enzyme in the generation, transport, and utilization of energy and has been well described by Huszar et al. (51,52). The sperm CK-M ratio represents the increased synthesis of the CK-M isoform that occurs during late spermiogenesis. Higher sperm CK activity signifies a defect of sperm development; in blinded studies, the CK-M ratio predicted the occurrence of pregnancies or the male fertility potential in couples using in vitro fertilization techniques (52,53).

Sample Size Calculations and Statistical Analysis

A priori sample size calculations were based upon the sperm measure with the greatest variability, namely, sperm concentration. Schrader et al. (54) cited a mean sperm concentration of 47.4 million per mL of semen having a within subject variability of 22.9 (S.D.). The present study was designed to have one baseline (pre-exposure) count followed by two additional measurements each approximately 15 weeks apart resulting in three measures per subject. With each person serving as his own control (pre–post measures), an exposure effect of one standard deviation, typical of most studies, with 80% power and an alpha of 0.05 was used to calculate a needed sample size of 20 subjects (55).

The assumptions of statistical analyses, including data normality and homogeneity of variances, were carefully examined, and the data were transformed as necessary to satisfy those assumptions. All sperm outcomes expressed as a percent were arcsine transformed. The use of dichotomous outcomes such as normal and abnormal was avoided because, according to Bigelow et al., subtle changes in semen variables possibly associated with workplace exposures may be detected only using continuous variables (56).

Job group was used as a surrogate measure of fuel and solvent exposure as well as a surrogate for other possible exposures occurring in the work environment and was coded and modeled as a "dummy" variable. Job descriptions and industrial hygiene measurements were used to group subjects by tasks. Evaluating subjects by job categories represents the activities performed throughout the time period and serves as an index of all exposures based on current job tasks. Total solvent and fuel breath levels were continuous variables used as a measure of internal dose in the multiple regression analyses. One breath sample value was missing for three individuals; and in these three instances the value obtain in the other cycle was substituted.

Possible correlations among the three biologic samples from the same individual were incorporated by performing a repeated measures analysis of variance. This analysis considers changes across time among the three samples related to exposure or other factors, and the variability among the subjects due to individual differences is removed from the error term (57). Factors considered as main effects in the model and collected with the baseline questionnaire included age (years), race (1 = Caucasian, 2 = otherwise), wearing briefs (1 = yes, 0 = no), usual frequency of hot baths (0 = never, 1 = less than once a month, 2 = 1 to 3 times per month), being a current smoker (1 = yes, 0 = no), and having had a sexually transmitted disease (STD) (1 = yes, 0 = no). Other potential main effects that were evaluated for time-dependent change at each cycle included reported fever of 101° or greater (1 = yes, 0 = no), number of cigarettes smoked per day, number of servings of coffee each day, number of servings of caffeinated beverages each day, servings of alcohol per month (0 = 0 to 1, 1 = 2 to 7, 2 = 8+), season of the year (1 = July/August, 0 = otherwise), and days of sexual abstinence before providing a sample (1 = <2 d, 0 = otherwise). Variables were chosen for the final repeated measures analysis if the variable was significantly ($P \leq 0.10$) associated with any semen outcome. Continuous or categorical variables of smoking, coffee, caffeinated beverages, and alcohol consumption were based on their association with semen outcome. The final model included age, race, smoking, having a STD, alcohol consumption, hot baths, and the season the sample was collected.

RESULTS

Subjects

Recruitment of volunteers required approximately 2.5 years. Of the 73 eligible subjects, 58 (79.5%) agreed to participate, which represents a high participation rate compared to other semen assay studies where participation rates are typically between 25% and 60% (26–28, 58,59). Age, race, and religion were similar in the participants and nonparticipant group, but average level of education differed as participants were more likely to have attended college (52% vs. 20%, $P = 0.04$). The participants were 74% Caucasian, 60% married, 66% of Protestant or Catholic religion, and relatively young, with a mean age of 26.5 years (range, 18 to 51). Only minor illnesses occurred during the 30 weeks. Nine reported fevers (≥101°F), two at baseline, and four and three during Cycles 2 and 3, respectively. The use of both prescription and over-the-counter medication was documented with only about 25% of the total group reporting medication usage other than a mild analgesic during each period.

Once the men began their work, job activities remained stable throughout the 30 weeks. The subjects were divided into five groups based on the type of job activity and probable exposure (Table 1). The *unexposed* group mainly performed office filing, typing, and clerical activities, and had no to minimal contact with industrial chemicals. Aircraft *sheet metal* workers performed assembly and maintenance activities and were exposed mainly to solvents, adhesives, and sealants, and also some purging fluid and jet fuel ($n = 6$). Aircraft *painters* were exposed to mainly solvents and paints ($n = 6$). The *jet fuels* group was mainly exposed to jet fuel (JP-4) and purging fluid; their duties consisted of fuel delivery, fueling and defueling aircraft, and repairing the fuel systems of F-16 aircraft ($n = 15$). The *flight line* crews were exposed to jet fuel and exhaust, solvents, and

Table 1. Demographics and lifestyle characteristics and possible risk factors among job groups at baseline values expressed as mean (standard deviation) or number (percent)

Demographic and risk factors	Not exposed *n* (%)	Sheet metal *n* (%)	Paint shop *n* (%)	Jet fuels *n* (%)	Flight line *n* (%)
Participants	8	6	6	15	23
Mean age (S.D.)[a]	26.0 (6.0)	34.5 (3.6)	31.7 (13.0)	24.1 (7.2)	24.8 (8.3)
Race:					
% Caucasian	3 (38)	4 (67)	5 (83)	12 (80)	19 (83)
% African-American	2 (25)	1 (17)	0 (0)	1 (7)	1 (4)
% Other	3 (38)	1 (17)	1 (17)	2 (13)	3 (13)
Cigarettes/d mean:					
None	6 (75)	6 (100)	5 (83)	9 (60)	13 (56)
1–19/d	2 (25)	0 (0)	0 (0)	3 (20)	4 (17)
20+/d	0 (0)	0 (0)	1 (17)	3 (20)	6 (26)
Alcohol, drinks/month					
0–1	3 (38)	1 (17)	2 (33)	6 (40)	9 (39)
2–7	3 (38)	2 (33)	1 (17)	4 (27)	2 (9)
>7	2 (25)	3 (50)	3 (50)	5 (33)	12 (52)
Servings of coffee/d					
None	3 (38)	0 (0)	1 (17)	9 (60)	8 (35)
1–2	4 (50)	3 (50)	3 (50)	5 (33)	10 (44)
≥3	1 (13)	3 (50)	2 (33)	1 (7)	5 (22)
Servings of caffeinated beverages/d					
None	1 (13)	0 (0)	1 (17)	1 (7)	0 (0)
1–2	2 (25)	0 (0)	0 (0)	6 (40)	5 (22)
≥3	5 (63)	6 (100)	5 (83)	8 (53)	18 (78)
Wears briefs					
Yes	7 (88)	5 (83)	4 (67)	13 (87)	18 (78)
Sexually transmitted disease (STD) Yes	2 (25)	0 (0)	3 (50)	4 (27)	3 (13)
Days of abstinence before semen sample					
≥2 d	7 (88)	6 (100)	5 (83)	15 (100)	23 (100)
Hot baths:[b]					
Never	2 (33)	1 (17)	3 (50)	8 (53)	13 (59)
<1 times/month	1 (17)	2 (33)	1 (17)	3 (20)	5 (23)
≥1 times/month	3 (50)	3 (50)	2 (33)	4 (27)	4 (18)
Season sample collected:					
Cool months 0	8 (100)	6 (100)	6 (100)	13 (87)	19 (83)
Hot months (July/August) 1	0 (0)	0 (0)	0 (0)	2 (13)	4 (17)

[a]Significant ($P = 0.01$) difference among groups (sheet metal versus fuels and flight line).
[b]One subject in flight line and tow unexposed did not provide information.

occasionally paint ($n = 23$), and included aircraft ground crews, jet engine mechanics, and those who worked on aircraft ground equipment and munitions equipment, both on jets parked in the shops and outside on the taxiway.

The demographics and potential risk factors by job group are summarized in Table 1. Only age was significantly different among job groups with the sheet metal group having a mean age of 24.5 compared to the jet fuels and flight line groups with mean age of 24.1 and 24.8, respectively ($P = 0.01$). The unexposed group was 38% Caucasian while the exposed groups ranged from 67% to 83%. All groups were predominately nonsmokers, the proportion ranging from 56% to 100%. Most had seven or fewer alcoholic drinks per month. All those working in sheet metal jobs drank coffee; the other groups had fewer coffee drinkers (40% to 66%). The study group generally wore briefs, ranging from 67% to 88%. The proportion of prior sexually transmitted disease (STDs) ranged between 13% (flight line crew) and 50% (painters).

Exposure Assessment Analysis (Industrial Hygiene Air Samples and Breath)

A total of 2,711 analyte values were obtained from the industrial hygiene sampling and 670 analytes were measured for the breath analysis. Exposures were low and well below the American Conference of Government Industrial Hygienists recommended threshold limit values (TLV) and those mandated by the Occupational Safety and Health Administration as personal exposure limits (PEL) (Table 2). All specific solvent IH measurements for the paint shop, and between 92% and 100% for the other three groups were less than 10 ppm (data not shown). Because most men were exposed to more than

Table 2. Mean solvent, fuel, and benzene exposure as measured by breath (ppb) and industrial hygiene (IH, ppm) by job activity reported as mean, range, and (SD and number of samples)

		Total solvents		Jet fuel as naphtha		Benzene[a]	
Job group	No of subjects	Breath (p.p.b.)	IH (p.p.b.)	Breath (p.p.m.)	IH (p.p.m.)	Breath (p.p.b.)	IH (p.p.m.)
All exposed	50	8.6 0.7–58.1 (10.6; $n = 94$)	1.6 0.0–106.9 (6.9; $n = 286$)	19.1 0.9–91.6 (18.9; $n = 53$)	3.2 0.0–163.1 (12.7; $n = 182$)	1.2 0.1–4.8 (1.1; $n = 53$)	0.05 0.0–1.7 (0.2; $n = 176$)
Sheet metal	6	24.0 5.3–58.1 (18.2; $n = 12$)	5.9 0.0–106.9 (17.5; $n = 36$)	28.3 6.3–91.6 (26.6; $n = 12$)	3.3 0.0–29.1 (5.9; $n = 36$)	0.7 0.1–2.1 (0.6; $n = 12$)	0.05 0.0–1.6 (0.27; $n = 36$)
Paint shop	6	10.3 4.0–28.4 (6.7; $n = 12$)	2.4 0.0–16.6 (4.3; $n = 35$)	13.1 7.5–16.0 3.3; $n = 6$)	1.4 0.0–7.9 (2.6; $n = 18$)	1.2 0.4–3.0 (1.2; $n = 6$)	0.0 0.0–0.0 (0.0; $n = 18$)
Jet fuel	15	5.9 0.9–44.4 (8.8; $n = 29$)	1.2 0.0–25.3 (3.7; $n = 89$)	16.8 1.1–63.0 (18.0; $n = 18$)	5.2 0.0–163.1 (20.1; $n = 68$)	1.1 0.1–3.3 (0.9–$n = 18$)	0.08 0.0–1.7 (0.27; $n = 62$)
Flight line	23	5.5 0.7–20.6 (3.9; $n = 41$)	0.46 0.0–11.8 (1.4; $n = 126$)	17.0 0.9–47.9 (15.5; $n = 17$)	1.5 0.0–13.7 (2.7; $n = 60$)	1.7 0.1–4.8 (1.4; $n = 17$)	0.02 0.0–0.2 (0.04; $n = 60$)

[a]A component of fuels and purging fluids and not shown separately hereafter.
OSHA Permissible Exposure Limit (PEL) and ACGIH recommended Threshold Limit Value (TLV) for individual solvents, benzene, and jet fuel as napthas are listed and identical unless reported otherwise. 1,1,1 trichloroethene = 350 ppm; MEK = 200 ppm; toluene 200 ppm PEL and 50 ppm TLV; methylene chloride = 500 ppm PEL and 50 ppm TLV; benzene = 1.0 ppm PEL and 0.5 ppm TLV; jet fuel as naphtha is 100 ppm PEL and unavailable for TLV.

one type of solvent and individual levels were consistently low, a "total solvent" value was derived by summing the concentrations of the analytes MEK, methylene chloride, xylenes, toluene, and TCA for the two exposure cycles. Mean industrial hygiene levels for total solvent and fuels were well below the threshold limit values (Table 2). Levels of total solvents, fuel, and benzene varied among the job groups. Fuels measured as napthas had a mean of 3.2 ppm, with a range from nondetectable to 163 ppm. Benzene, a component of fuels and recycled purging fluids had mean levels below detection for those in the paint shops where fuels are not used, ranging up to a mean level of 0.08 ppm for those performing jet fueling operations.

Breath measurements of solvents showed no unusually high peaks of exposure across periods, and were comparable for both cycles. The pooled data are shown in Table 2. For all exposed subjects, the mean breath level of total solvents and fuels was 8.6 ppb and 19.1 ppb, respectively. Sheet metal workers showed the highest mean breath levels for total solvents (24.0 ppb) and fuels (28.3 ppb), whereas the flight line crew, who primarily worked outdoors, had the lowest total solvent exposure (5.5 ppb) and the paint shop had the lowest fuel exposure (13.1 ppb). Generally, internal dose levels of solvents and fuels were stable. There was a significant decrease in breath fuel levels in cycle three versus two for flight line personnel ($P = 0.004$) and sheet metal workers ($P = 0.04$). It should be recalled, however, that these differences represent acute exposure and are related to tasks performed on the day of sampling.

Semen Outcomes

The findings for semen measures, by cycle, for all exposed subjects are compared to reference values provided by the World Health Organization (60) or published studies of nonexposed subjects (50,52,54,61) (Table 3). Table 3 is descriptive demonstrating that the semen parameters at all cycles were similar to reference limits, except for percent motile, which was consistently lower. Percent motile is dependent on time from postejaculation until sample analysis. The average time elapsed was 52.4 (S.D. 21.4) min over all three sampling periods, and no significant time difference was found by cycle. The eight *unexposed* subjects (values not shown) also were similar to the exposed group as well as the reference values, and percent motile sperm was also lower at baseline in this group than the reference.

Changes in semen parameters between the baseline and the first and second cycles were evaluated using a multiple regression repeated measures analysis (62). Exposure variables included an analysis by job group and internal dose levels measured as either total solvents or fuels. This analysis tested each exposure variable individually, and also total solvent and fuel were modeled combined and with interaction terms. Exposure as measured by total solvent level was not significant for any change in semen parameters, and fuel exposure was associated with a change in only one outcome, percent viable by dye exclusion. Therefore, the findings are shown for the job group analysis only.

The results of the repeated measures analysis by job group adjusted for other significant covariates are pro-

Table 3. Descriptive values for semen parameters for the combined exposed group ($n = 50$) at baseline, 15, and 30 weeks given as mean and (SD)

	Baseline	15 weeks exposure	30 weeks exposure	Reference values[b]
Sperm, production, function, and movement:				
Sperm concentration (million per mL)[a]	66.4 (32.6)	72.4 (46.9)	73.8 (47.7)	≥20 (60)
% Viable by dye exclusion[a]	75.4% (8.8)	73.7% (13.0)	75.8% (10.2)	≥75% (60)
% Viable by swelling	69.8% (11.3)	71.7% (12.6)	73.1% (9.4)	≥64.1% (54)
% M-type creatine kinase	24.3% (17.2)	23.5% (15.5)	21.5% (15.78)	≥10% (51)
% Motile sperm[a]	44.5% (12.0)	43.7% (14.9)	42.0% (12.3)	≥50% (60)
Straight line velocity (VSL)	40.9 (11.0)	40.6 (11.9)	39.3 (10.1)	N/A
Curvilinear velocity (VCL)	67.8 (10.6)	69.7 (10.2)	68.0 (8.8)	N/A
Linearity (VSL/VCL)	5.9 (0.8)	5.2 (1.1)	5.7 (0.8)	5.8 (61)
Mean ALH displacement	4.0 (0.3)	4.1 (0.4)	4.1 (0.4)	N/A
% Normal morphology (not strict)[a]	51.7% (10.9)	51.1% (13.4)	50.2% (12.2)	≥30% (60)
% Normal morphology (strict)[a]	18.4% (6.6)	17.8% (8.6)	18.1% (9.1)	≥14% (60)
Morphometry:				
Length (μm)	4.26 (0.28)	4.25 (0.27)	4.21 (0.25)	4.5 (63)
Width (μm)	2.81 (0.16)	2.81 (0.16)	2.76 (0.17)	2.9 (63)
Area (μm)	8.56 (0.84)	8.57 (0.86)	8.34 (0.89)	8.7 (63)
Perimeter (μm)	11.65 (0.62)	11.64 (0.63)	11.50 (0.62)	12.7 (63)
Width/length	0.67 (0.05)	0.67 (0.04)	0.67 (0.04)	0.6 (63)
SCSA outcomes:				
±Native DNA stain (Green)	403.86 (44.0)	402.90 (41.0)	411.94 (42.5)	506.2 (29.8) (50)
± $alpha_t$ (total stain)	267.70 (64.4)	265.62 (54.2)	255.67 (43.4)	222.3 (38.9) (50)
Standard deviation $alpha_t$ (total)	155.61 (51.6)	154.34 (49.5)	147.11 (44.0)	155.3 (38.7) (50)
% Cells DNA denatured (COMP $alpha_t$)	18.3 (11.9)	19.0 (11.4)	17.1 (9.3)	16.8 (7.2) (50)

N/A = not available since dependent on software system and frame speed.
Slightly different numbers of samples during the three periods attributable to accidental loss or damage and three subjects withdrew after the second sample.
[a]Traditional clinical assessment measures.
[b]Reference values for WHO are consensus numbers, other values may be laboratory-specific; only to be used as guide for interpretation. Those values presented for linearity, the morphometry, and SCSA measures represent mean values found in unexposed groups, whereas, the other values for sperm production, function and movement, are lower limits estimated for "normal" functioning.

vided in Table 4. When job group was the surrogate measure for exposure, several outcome parameters were significant. Job group was associated with a change in sperm concentration, measures of sperm motion (percent motile and velocity as measured by linearity and straight line motion), and measures of sperm morphology (sperm length and the ratio of sperm width divided by the length). The next analysis focused on which job groups were associated with these changes. Patterns varied among the job groups (Table 5). The flight line group demonstrated a significant increase of 32.9% ($P = 0.02$) in sperm concentration at 30 weeks. Sperm length demonstrated a significant ($P = 0.02$) 2.1% and 2.9% decline at 15 and 30 weeks in the sheet metal group, but a significant ($P \leq 0.01$) 2.5% decline at 15 weeks also was found in the unexposed group. The sperm width to length ratio also significantly declined in the unexposed ($P = 0.05$) and the paint shop ($P = 0.02$) groups at 30 weeks. Sperm directional movement as measured by linearity (VSL/VCL) was significantly depressed at 30 weeks in the sheet metal (8.8%, $P = 0.03$) and the fuels (7.7%, $P = 0.02$) groups.

Sperm motility showed the sharpest average decline (19.5%, $P = 0.04$) at 30 weeks for the men working in the paint shop. Five of the six painters showed a percent decline in motility, ranging from 3.5% to 43.7% (Table 6). In a previous report, significant increases in genotoxic measures as evaluated by the sister chromatid changes (SCEs) and micronuclei (MN) assay also were found in this same group of painters (34). The painters showed an increase in SCEs from 5.9 to 6.7 ($P = 0.05$), which correlated well ($r = -0.56$, $P = 0.06$) with their sperm motility decline, and as shown in Figure 1, except for painter B, all showed a greater decline in sperm motility but an increase in SCEs from 15 to 30 weeks.

DISCUSSION

Exposures to solvents and fuels were generally low for all subjects. The sheet metal group had the highest mean internal breath exposures to both solvents and fuels. Since benzene is a carcinogenic component of JP-4, it was of particular interest. Benzene levels in the breath ranged between nondetectable and 4.8 ppb. Average IH samples for benzene ranged from below the limit of detection to 1.7 ppm, sometimes exceeding the ACGIH TLV of 0.5 ppm and the OSHA PEL of 1.0 ppm. After this study ended, the USAF converted to JP-8, which has a lower benzene content.

Neither body burden measures of solvents nor fuels

Table 4. Repeated measures analyses of baseline, 15-, and 30-week cycles for all subjects ($n = 58$) by job group adjusted for significant covariates for each semen outcome

Outcome and significant variables	Coefficient	95% C.I.	*P*-value
Sperm Production, Function, and Structure:			
Sperm Concentration (million/mL)			
Job group	N/A		<0.01
% Viable by dye exclusion			
Smoking	0.071	0.016, 0.126	0.01
% Motile sperm			
Job group	N/A		0.02
Hot baths	0.095	0.016, 0.144	<0.01
Race	0.071	0.019, 0.123	<0.01
Smoking	0.075	0.024, 0.125	<0.01
% Normal morphology (strict)			
Smoking	0.047	0.017, 0.077	<0.01
Straight line velocity (VSL)			
Job group	N/A		<0.01
Hot baths	3.728	0.249, 7.207	0.04
Season	−6.779	−11.019, −2.540	<0.01
Smoking	5.040	1.453, 8.626	<0.01
Curvilinear velocity (VCL)			
Hot baths	4.203	1.005, 7.401	0.01
Season	−5.442	−9.339, −1.545	<0.01
Smoking	5.425	2.128, 8.722	<0.01
Linearity (VSL/VCL)			
Job group	N/A		<0.01
Season	−5.111	−8.920, −1.303	<0.01
Mean ALH displacement			
Alcohol	N/A		0.04
Abstinence	0.005	0.002, 0.008	<0.01
Smoking	0.146	0.014, 0.277	0.03
% M-Type creatine kinase			
Age	0.008	0.004, 0.011	<0.01
Race	−0.103	−0.168, −0.038	<0.01
STD	−0.089	−0.162, −0.015	0.02
% Normal morphology (traditional)			
Smoking status	0.099	0.048, 0.149	<0.01
Morphometry:			
Length (μm)			
Job group	N/A		0.01
Alcohol	N/A		0.03
Race	0.159	0.060, 0.258	<0.01
Smoking	−0.118	−0.214, −0.022	0.02
Width (μm)			
Smoking	0.061	0.004, 0.118	0.04
STD	−0.079	−0.145, −0.014	0.02
Area (μm)			
STD	−0.479	−0.836, −0.122	<0.01
Perimeter (μm)			
Race	0.314	0.078, 0.550	0.01
Width length (μm)			
Job group	N/A		<0.01
Race	−0.021	−0.036, −0.005	<0.01
Season	−0.020	−0.038, −0.002	0.03
Smoking	0.032	0.017, 0.047	<0.01
SCSA Outcome:			
$\bar{\times}$ Native DNA stain (green)			
Age	−1.380	−2.209, −0.550	<0.01
$\bar{\times} a_t$ (Total stain)			
Age	1.911	0.872, 2.951	<0.01
Race	−22.986	−41.131, −4.841	0.01
SD a_t (total)			
Age	1.797	0.867, 2.727	<0.01
Race	−17.912	−34.143, −1.681	0.03
Smoking	−23.189	−38.925, −7.454	<0.01
% Cells DNA denatured (COMP a_t)			
Age	0.005	0.003, 0.007	<0.01
Race	−0.044	−0.082, −0.006	0.02
Smoking	−0.041	−0.078, −0.004	0.03

N/A = not applicable for categorical variable; subsequent pairwise comparison and significant pair(s) reported in Table 5.
STD = sexually transmitted disease.
% viable by swelling had no significant risk factors.

Table 5. Proportional (percent) increase (+) or decrease (−) from baseline * at 15- and 30-week cycle by job group for significant findings from repeated measures analysis (previous table)

Outcomes	Cycle (weeks)	Unexposed	Sheet metal	Paint shop	Jet fuel	Flight line
Sperm concentration (million/mL)	15	+1.4	−18.3	+33.4	+9.7	+34.0[e]
	30	+23.7	−19.5	+43.8	+9.0	+32.9[d]
Sperm length (μm)	15	−2.5[e]	−2.1[d]	−1.2	+1.5	−0.3
	30	−1.1	−2.9[d]	0.0	+0.8	−1.6
Sperm width/length	15	−1.5	+0.6	+1.0	+1.1	−0.4
	30	−3.1[a]	+2.0	−3.4[d]	+0.1	−1.2
% Motile sperm	15	+15.9	−4.6	−6.4	−2.1	+2.9
	30	+8.1	−3.2	−19.5[b]	−6.2	+7.2
Straight line velocity (VSL)	15	−5.7	−10.6	+17.8	+3.0	+1.2
	30	+0.7	−8.7	+4.0	−7.8	+2.6
Linearity (VSL/VCL)	15	−5.1	−8.9	+2.2	+0.2	−4.3
	30	−3.9	−8.8[c]	−0.7	−7.7[d]	0.0

*[(week 15 or 30 − baseline)/baseline] × 100
[a]$P = 0.05$ using paired t test.
[b]$P = 0.04$
[c]$P = 0.03$
[d]$P = 0.02$
[e]$P = 0.01$

were associated with spermatogenic changes, but significant associations were found when exposure was assessed by job group. There may be several explanations for these differences in findings by exposure model. The first is that these mixtures may not be associated with spermatotoxic effects at low levels, and the findings related to job groups may be an artifact. Another explanation is that exposure measured at one point in time may be inadequate for characterizing "true" absorbed dose. Breath samples are a measure of exposure similar to a one time blood draw, and interpretation of exposure concentration depends on timing of the exposure and clearance times. For the targeted chemicals, the clearance time was between minutes and 1 to 2 h. Our procedures allowed the worker to "off-gas" for 1 h before sample collection in order to reach steady-state. This procedure may have limited our ability to detect "true" exposure levels because of clearance of the compounds. Finally, though we sought to measure the most ubiquitous and potentially hazardous workplace exposures, it was shown in our pilot investigation that between 50 and 100 chemicals can be measured in the breath of aircraft maintenance workers (36). Therefore, job group may have more accurately reflected the myriad of exposures than did the measured compounds.

As noted in Tables 4 and 5, significant results were found by job groups. Sperm concentrations significantly increased over the 30 weeks for the flight line group and there was an upward trend for three other groups. This finding is likely related to measurement variability since this outcome is inherently variable and there was only

Table 6. Sperm motility changes from baseline for the painters

Painter ID	Baseline (%)	Cycle 1 (%) (15 weeks)	Cycle 2 (%) (30 weeks)	Proportional change (%)[a]
A	56.7	56.1	54.7	−3.5
B	45.5	42.7	31.7	−30.3
C	40.6	51.0	42.0	+3.5
D	31.6	27.7	23.8	−24.7
E	41.4	25.9	23.3	−43.7
F	62.6	58.2	51.0	−18.5

[a][(Week 30− Baseline) Baseline] × 100).

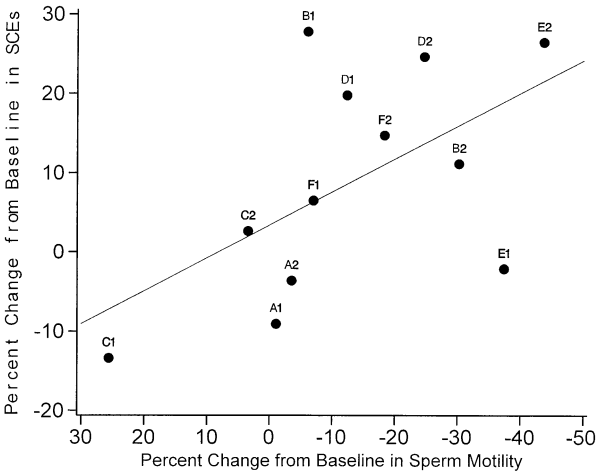

Fig. 1. Percent changes in sperm motility and sister chromatid exchanges (SCEs) for the painters, identified alphabetically as in Table 6 at 15 (1) and 30 (2) weeks.

one sample collected at each time period (63). Having each individual serve as his own control by collecting a pre-exposure sample should help to control for intra-individual variability attributable to heredity, lifestyle, and other factors. When biologic measurements are highly variable, however, one baseline value may have been insufficient to adjust for a high degree of test variance. For this study, the inclusion of a completely unexposed group aided interpretation of these findings by adding a reference point for evaluating measurement variability. In Table 5 it was shown that the unexposed, sheet metal, and paint shop groups all had a significant decline in one of two measures of sperm morphometry. The findings in the unexposed group raise questions about the importance of sperm morphometry measures in the sheet metal and paint shop groups.

For measures of sperm movement, both sheet metal and jet fuel groups showed a significant decrease in linearity at 30 weeks, and the paint shop had a significant decline in percent motile. A study limitation, however, is that these significant findings may be due to multiple comparisons as a large number of endpoints are typically measured in male reproductive studies. Savitz and Olshan (64,65) and others (66,67) suggest that in these instances each hypothesis should be evaluated by the quality of the results and the compatibility of the findings with other evidence, particularly when little is known about biologic mechanisms and exposure pathways as is the case for most reproductive toxicants. Because of the nature of the outcome in question, it is unclear as to how much "effect" constitutes a deleterious or harmful effect, and in these instances a flexible approach in analysis and interpretation may be warranted (64,65,67).

Another study limitation may be related to low statistical power. As described earlier, the targeted sample size was 20. The combined category of aircraft maintenance workers totaled 50 subjects, two and a half times the target. As shown in Table 2, however, exposures varied amongst the subgroups. For examination of effects by specific job group, the sample size was adequate for the flight line crews ($n = 23$), marginal for the jet fuel operators ($n = 15$), and small ($n = 6$) for the sheet metal and painter groups. Hence, lack of significant findings in three of the four groups may be attributable to low power, which may mask a potential health effect (68,69).

On the other hand, it may be illuminating to further investigate positive findings in analyses unlikely to be significant, i.e., those having low power. In particular, we feel that the finding warranting further evaluation is related to the decline in average sperm motility of 19.5% for aircraft painters; this was the largest significant decrease, followed next by a proportional decrease of 8.8% in linearity, another measure of movement. Sperm motion parameters are stable, precise, and not easily subjected to slight environmental perturbations (61) and may be one of the most important indicators of fecundity. Studies have shown that the percentage of motile sperm is positively correlated with pregnancy rate (70), successful in vitro fertilization rates of human oocytes (71,72), and the number of living children (73). Sperm motility also is a sensitive target for reproductive toxicants. Studies using Swiss mice showed that 89% of the known male reproductive toxicants caused significant decreases in sperm motility (74). As noted in Table 5, the paint shop group had a decrease in motile sperm but the straight line velocity (VSL) and curvelinear velocity (VCL) showed a nonsignificant positive increase. This apparent contradictory relationship may be an artifact, however, as recently documented in a rabbit study of sperm toxicity related to lead exposure (75). In that study, velocities also showed a dose-dependent increase until adjusted for the decrease in the distribution of motile cells (velocity $\times$ percent motile/100); then a significant decrease in all velocity measures (VSL, VCL, and average path velocities) became apparent. When we made this adjustment for the painters, a proportional decrease of 18% ($P = 0.09$) for VSL and a 17.7% ($P = 0.05$) for VCL was also found.

The sheet metal group compared with the painters had higher fuel and solvent exposures but the decline in sperm motility was only 3.2%, compared with 19.5% for the painters. This lack of a dose–effect relationship is troublesome. The painters, however, did have higher breath benzene levels and also were exposed to a wide variety of compounds not measured in this study. The possibility exists that unmeasured agents such as glycol ethers that have been associated with painting operations and also associated with male reproductive effects (27) may account for the decline in motility. Our pilot study of three aircraft painters demonstrated that they had between 50 and 100 measurable compounds in their breath (36). During actual painting operations, painters usually wore half-face cartridge respirators. It was our observation, however, that, after completion of the spray operations, while often still in the paint booth, the protective respiratory protection is removed, and they may breathe vapors remaining in the air. Certainly, other occupational health studies have shown that painters are a potentially high-risk group with adverse health findings demonstrated for neurologic changes (76,77), bladder cancer (78–83), and lung cancer (84–88) among other conditions.

In conclusion, our findings indicate that exposure to jet fuel did not show an apparent effect on semen quality for aircraft maintenance personnel. A subgroup analysis of those men involved in aircraft painting operations with minimal fuel exposure, but higher exposure to

solvents suggests the need for further investigation. Because of the aforementioned study limitations, the lack of significance for the smaller subgroups could be related to low power, and the positive findings may also be related to inherent variability in the measures or to chance.

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